

Morphological and Phylogenetic Studies of Fusarium species in Iran

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ABSTRACT: From 1200 isolates of *Fusarium* which from different plant organs gramineous plant including crown, root, stem and spike on conventional PDA and specific Nash & Snyder media were obtained, 35 species of *Fusarium*, were identified based on morphological characteristics. From isolates obtained 30 isolates selected as representative molecular studies were reviewed. The results of this study showed, isolates in seven phylogenetic groups (clades) were used. Isolates of *F. nygamai*, *F. pseudonygamai*, *F. proliferatum* var. *proliferatum*, *F. proliferatum* var. *minus* and *F. fujikuroi* isolates formed a monophyletic group, *F. solani*, *F. tricinctum*, and *F. sporotrichioides*, *F. pallidoroseum*, *F. semitectum* and *Fusarium* sp. every one constituted into another monophyletic groups. As predictable is based on morphological characteristics of isolates of *F. nygamai* were two *Liseola* and *Elegans* sections.

Keywords: Morphological, Phylogenic, *Fusarium*, Gramineous Plants, Iran

INTRODUCTION

The *Fusarium* genus is hyphomycetes soilborne fungi which includes many species that has high economic importance of plant diseases and has a wide range of host plants, such as tomatoes, potatoes, legumes, clove and grasses such as wheat, barley, oats, maize and sugarcane many of the infected vegetables and other plants (Nelson et al., 1983, Burgess et al., 1994). Species of this genus have a wide distribution in all regions of the world and many of it's species as chlamydospores in soil texture or passive and active mycelium in host debris and organic material present (Burgess et al., 1994, Summerell et al., 2003). *Fusarium* species pathogenic on plants and products, in addition to are the main cause of pollution mycotoxicoses in humans and other animals (Monds et al., 2005, Nelson et al., 1993). Some *Fusarium* species such as *Fusarium verticillioides* (= *F. moniliforme*) to cause seedling blight, root and stalk rot of corn and corn products decreased on average by 8-4% percent on year, and also are causing disease in corn, bananas, figs, pine, rice and sorghum (Nelson et al., 1993). With the advent of molecular techniques, a new stage of *Fusarium* taxonomy is controversial. Phylogenetic species concept and it's practical application so that especially the combination of these concepts based on DNA data in the past few years has led to the emergence of new species. On the other hand, phylogenetic and population genetics methods that compare the changes in nucleic acids are used for identification of the fungal pathogen population and how their natural reproduction. Also the molecular tools has accelerated accuracy of in the development and classification of unknown *Fusarium* isolates (Leslie et al., 2001; Tylor et al., 2000; Tylor et al., 1999; Arie et al., 1997; O'Donnell, 1996). On the other hand, several molecular tools such as AFLPs (amplified fragment length polymorphisms), RFLPs (restriction fragment length polymorphisms), RAPD (random amplified polymorphic DNA), PFGE (pulse field gel electrophoresis), SSCP (single-strand conformation polymorphisms), isoenzyme (isozymes), hybridization of DNA-DNA, probes DNA, protein electrophoresis, Microsatellite markers (microsatellite markers) sequenced portions of the genes 18S, 28S, (internal transcribed spacer) ITS, (intergenic spacer) IGS, rDNA, the small subunit ribosomal DNA and mitochondrial ribosomes and nuclear gene sequences such β -tubulin, calmodolin and EF-1 α (Translation Elongation Factor) is applied to measure and evaluate the diversity of microorganisms and their classification in all surfaces taxonomic such as *Fusarium* (Bruns et al., 1990; Partridge, 1991; Donaldson et al., 1995; Parry and Nicholson, 1996; O'Donnell, 1998; Hyun and Clark, 1998; Yoder and Christianson, 1998; Abdel-Satar et al., 2003; Waalwijk et al., 2003; Prasad et al., 2004; Mirete et al., 2004; Moretti, et al., 2004; Gonzalez-Jaen, et al., 2004).

MATERIALS AND METHODS

Isolation and Identification

In this study, samples of plants infected and suspicious of gramineous plants and pasture grasses from different parts of the country, were collected in a paper bag (not used) and transferred to the laboratory. After transferring the samples to the laboratory, various organs of host plants including root, crown, stem and spike were cultured on a specific medium modified Nash and Snyder (Burgess et al., 1994), and common medium potato - dextrose agar (PDA) (Nelson et al., 1983; Burgess et al., 1994). For identification of *Fusarium* species, isolates single sporing were on different media (PDA, CLA, SNA, KCl and SA) and were taken on light conditions and temperature. Then, they was identified by using the keys and papers that have been published recently and color chart (Booth, 1971; Nelson et al., 1983; Gerlach and Nirenberg, 1982; Burgess et al., 1994; Nirenberg and O'Donnell, 1998; O'Donnell et al., 2004; Leslie and Summerell, 2006).

Biomass preparation and DNA extraction

For biomass production, in petri dish plate 15-10 ml of sterile PDA was poured and after cooling put into a 9 cm diameter sheet were sterilized opp (cellufan). Then mycelium of cultured isolates collected 5 days by scalpel and placed into a tube eppendorf 1.5 ml and transferred to a 1.5 ml of fungal suspension prepared in sterile water and a drop of Tween -20 (10%) with a final concentration of 0.05 percent added using a micropipette one ml of the suspension was poured on the sheet opp. And by sterile L-shaped glass tube was broadcast by a temperature of 25 ° C was maintained in dark conditions. After 5-4 days the mycelium sterile scalpel sliced dry sterile Whatman paper and OPP collected cc15 Falcon tubes were placed in a freezer at - 70 ° C. The extracted DNA was stored. The 0.5 grams of fungus mycelium in liquid nitrogen and placed in a sterile porcelain mortar and pestle and add to the flour and rub until completely disintegrated fungal tissue. Then sterilize a metal palette knife and roll it into a tube eppendorf 1.5 ml was poured into 400 ml of saline DNA (DNA salt solution) with compounds (100 mM, Tris-HCl; 5mM, EDTA; NaCl 1.4 M pH 7.5-8.0) was added and vortexed until the suspension was completely at 65 ° C water bath for 15 min. Then eppendorf tubes were placed on ice for 5 min. The tubes were centrifuged for 10 min (Allegra 64R Centrifuge, Beckman Coulter Inc. USA) at 4 ° C and were centrifuged at a speed of 13,000 rpm. Residual cells and proteins precipitated and the supernatant containing the DNA was transferred to a new tube eppendorf. The 7/0 alcohol volume of isopropanol was added and vortexed gently until the DNA is completely dissolved. The tubes are then centrifuged for 15 min (Eppendorf Centrifuge 5415D, Germany) with a speed of 10000 rpm at room temperature was placed. Supernatant was discarded and the DNA precipitated at the bottom of the tube on a clean paper towel or dryer sheet was upside down to dry. After drying, the dose of 100 micro liter of sterile distilled water or buffer twice TE (TE buffer; 10 mM, Tris-base pH 8, 1mM, ETDA pH 8) was added and kept in the refrigerator for 24 hours (overnight) and the DNA was completely resolved for future studies in the freezer at - 20 ° C was maintained After drying, the dose of 100 micro liter of sterile distilled water or buffer twice TE (TE buffer; 10 mM, Tris-base pH 8, 1mM, ETDA pH 8) was added and kept in the refrigerator for 24 hours (overnight) and the DNA was completely resolved for future studies in the freezer at - 20 ° C was maintained (Cenis, 1992; Duteau and Leslie, 2005).

Determine the quality and quantity of DNA

Nucleic acid and protein-nucleic acid ratio in each sample was performed using a Beckman DU 530 spectrophotometer and Eppendorf Biphotometer, Germany. At first a cuvette crystal machines was cleaned with acetone and devices were adjusted at zero with sterile distilled water (blank). Then four-microliter DNA samples extracted were diluted with 596 ml of sterile distilled water (1:150) was infused in cuvette. The optical density (OD) was measured at 260 nm and protein-nucleic acid at 280 nm. Light absorption was read at a wavelength of 260 nm to the absorbance at 280 nm from the device. If the ratio was in the range 1.8-1.9, indicating that nucleic acids have been attracted solely by the desired amount and has a good quality and it was used in later works of otherwise specimens removed and re-extracted DNA was done from the samples. Gene amplification of TEF (Translation Elongation Factor).

Part of the TEF gene amplification was performed using two primers ef1 and ef2 (O'Donnell and Cijelnik, 1997). PCR reaction volume was prepared of 50 ml containing 38 ml of sterile distilled water, 5 ml of buffer PCR, 1.5 ml 50 mM magnesium chloride (MgCl₂, Merck), 1 ml mixture of nucleotides dATP, dTTP, dGTP and dCTP (200 mM), 2 ml of fungal DNA (10 ng), 2 ml mixture of primers (20 mM) and 0.5 ml enzyme, Taq polymerase (5 units per ml) (Ampli Taq Polymerase, Cinna Gen). Tubes in the PCR (Master Cycler Eppendorf Germany) was used and the reaction program consisted of 4 min at 94 ° C, 35 cycles of 35 seconds at 94 ° C, 55 seconds at 52 ° C, 2 min at 72 ° C and final 10 min at 72 ° C was performed. After completion of the reaction rate of 5 ml of PCR product was mixed with 3 ml of loading buffer into the wells caused by agarose gel was empty. Action Electrophoresis was performed for 30

min at constant current 60 mili amp (O'Donnell and Cijelnik, 1997). Marker 1kb (DNA Ladder, GIBCO, BRL) in the wells of the gel with samples of 3 ml were empty.Amount of 5 ml of the master mix with 3 ml loading buffer and gel at final well as the negative control was load. After electrophoresis, the gel under UV (254 nm) was observed and imaged with the camera (VILBER LOURMAT 77202 MARNE LA VALLE cedex France) and was saved using the Gel Doc. The experiments were successful in doing multiplication and division as a distinct band was amplified, PCR product for subsequent studies was maintained in a freezer at - 20 ° C (Figure 1).

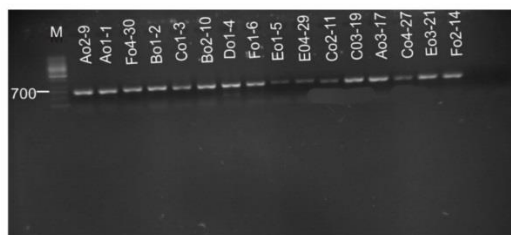


Figure 1. Size of sections amplified by primers ef1 and ef2

Purified DNA was amplified using specific purification kit (AccuPrep PCR DNAPurification Kit, K-3034-1, Bioneer Corporation).

First, 48 ml of absolute ethanol was added to wash buffer (Washing Buffer). Five volumes of buffer PB (PCR Binding Buffer) were added to one volume of PCR product (45 ml PCR product was removed and the volume was brought to 225 ml) and mixed thoroughly. Then a column of Binding was inserted within a 2-ml tube of eppendorf and sample was poured into the column. Sample were centrifuged with rate of 13,000 rpm at room temperature. Excess solution was discarded and 500 ml washing buffer (WB) was added to the column and was centrifuged for a minute speed at 13,000 rpm at room temperature. Then excess solution was discarded and the second column was inserted in a 2 ml tube of eppendorf and add 500 ml washing buffer (WB) and were centrifuged for a minute at 13,000 rpm speed at room temperature. Binding column was put within a 1.5 ml tube of eppendorf and a 30 microliter samples were inserted TE buffer or sterile distilled water two times a minute in the middle column of Binding placed on a metal rack were to remain constant. Binding column and tube was centrifuged for a minute at a speed of 13,000 rpm. Then the column was removed and the DNA purified product was collected in tubes at - 20 ° C freezer were stored for subsequent studies (Figure 2).

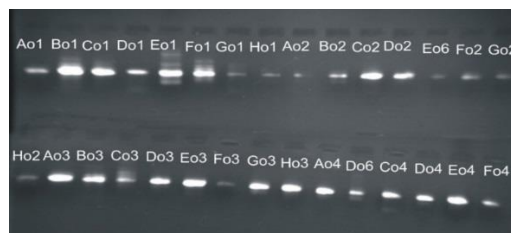


Figure 2. PCR product was purified using purification kit, Bioneer

DNA amplified sequencing by primers EF1 and EF2

The sequencing of the amplified using two primers, one-way operation sequencing was used by primers ef22 to Forward with Sequencer (3130x1 genetic analyser) Co ABI (16 capillar) (Table 1).

Primers	Sense	Sequence
EF1	Forward	5'-ATGGGTAAGGAR(C/G)GACAAGAC-3'
EF2	Reverse	5'-GGARGTACCAAGTS(T/A)ATCATG-3'
EF22	Reverse	5'-AGGAACCCTTACCGAGCTC-3'

Sequencing was performed in a sequence using gene sequences from Gene Bank (NCBI) arose out (alingment) took the field

Alingment sequences and phylogenetic analysis

To align sequences using GeneDoc 2.6.002 software (Nicholas and Nicholas, 1997) was adjusted first automatically and then manually. Phylogenetic analysis of the Neighbor-Joining method were performed using the software (Thompson et al., 1997) Clustal X and Treeview version 0.4 (R. Page, 2001) and with thousands of Bootstrap replicates.

RESULTS AND DISCUSSION

Results

Gene TEF (Translation Elongation Factor) is a protein that is translated into an essential part of the encoding is high phylogenetic application because: 1 - the data shows a high level of *Fusarium* species. 2- No non-identical copies of (non-orthologous) genes are made of this genus. 3- The general primers were designed to introduce and operate at the genus level (Geiser et al., 2004). This gene first was used as a marker for species identification and phylogenetic relationship of the genus night blades Order Lepidoptera by Cho and colleagues (Cho et al., 1995). In this study, the generation of primers was used for genes in fungi (lineage) of *Fusarium oxysporum* was used first by O'Donnell and colleagues (O'Donnell et al., 1998c). Ef1 and ef2 primer on some of the exons applied in *Trichoderma reesei* and *Histoplasma capsulatum*, and these primers can be used in wide varieties of filamentous ascomycetes. The primers that amplify a region of 700 bp intron have been located in all three regions around known *Fusarium* species (Figure 3).

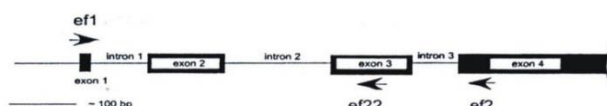


Figure 3 . Map of the TEF gene regions in *Fusarium* is used

Single copies of this gene are found in the genus *Fusarium* and demonstrated high levels of sequence polymorphism and even in comparison with protein coding genes such as calmodulin, β -tubolin and histone H3 in the species that are relatively close (Geiser et al., 2004) and were selected as a tool for determination of TEF (single-locus) in the genus *Fusarium*. In this study section TEF1, TEF2 and TEF22 some strains of the genus *Fusarium* were amplified and sequenced.

The length and sequence of DNA segments amplified with primers ef-1 and ef-2

Morphological and molecular studies of 30 isolates that the other species have been identified or suspected, as well as a representative number of isolates molecular sequencing studies were identified (Table 2).

No	Isolates	Species	Host	Locality
1	F-373-1 (Ao1-1)	<i>F. nygamai</i>	barley	Yazd - Bafgh
2	F-740 (Bo1-2)	<i>F. proliferatum</i>	barley	Mazandaran - Behshar
3	F-453 (Co1-3)	<i>F. nygamai</i>	barley	Yazd - Bafgh
4	F-347 (Do1-4)	<i>F. solani</i>	Wheat	Yazd - Bafgh
5	F-t (Eo1-5)	<i>F. tricinctum</i>	Wheat	Fars - Marvdasht
6	F-1087 (Fo1-6)	<i>F. proliferatum</i>	corn	Zanjan
7	F-278 (Go1-7)	<i>F. nygamai</i>	barley	Kerman - Bezanjan
8	F-318 (Ho1-8)	<i>F. nygamai</i>	barley	Yazd - Harat
9	F-492 (Ao2-9)	<i>F. nygamai</i>	wheat	Kerman - Orozoieh
10	F-1094 (Bo2-10)	<i>F. proliferatum</i>	wheat	Zanjan
11	F-261 (Co2-11)	<i>F. nygamai</i>	wheat	Kerman - Orozoieh
12	F-393-1 (Do2-12)	<i>F. nygamai</i>	barley	Kerman - Orozoieh
13	F-777 (Eo2-13)	<i>F. pallidoroseum</i>	aegilops	Mazandaran
14	F-361 (Fo2-14)	<i>F. nygamai</i>	wheat	Fars - Kachuyeh
15	F-429 (Go2-15)	<i>F. solani</i>	barley	Yazd - Bafgh
16	F-373 (Ho2-16)	<i>F. nygamai</i>	barley	Yazd - Besez
17	F-563 (Ao3-17)	<i>F. chlamydosporum</i>	wheat	Fars - Saadatshahr
18	F-1196 (Bo3-18)	<i>F. fujikuroi</i>	rice	Gillan - Lahijan
19	F-393 (Co3-19)	<i>F. nygamai</i>	wheat	Kerman - Orozoieh
20	F-157 (Do3-20)	<i>F. pseudonygamai</i>	sugarcane	Khuzestan - Karun
21	F-540 (Eo3-21)	<i>F. nygamai</i>	wheat	Kerman - Orozoieh
22	F-365 (Fo3-22)	<i>F. cf. semitectum</i>	wheat	Fars - Niriz
23	F-897 (Go3-23)	<i>F. proliferatum</i>	wheat	Kohgiluyeh - Dalyn
24	F-840-1 (Ho3-24)	<i>Fusarium sp.</i>	oat	Golestan
25	F-1199 (Ao4-25)	<i>F. fujikuroi</i>	rice	Gillan - Lahijan
26	F-536 (Bo4-26)	<i>Fusarium sp.</i>	wheat	Kerman - Mahan
27	F-1201 (Co4-27)	<i>F. oxysporum</i>	rice	Gillan - Lahijan
28	F-313 (Do4-28)	<i>F. oxysporum</i>	wheat	Kerman - Dehroo
29	F-719 (Eo4-29)	<i>F. sporotrichioides.</i>	wheat	Golestan
30	F-344-1 (Fo4-30)	<i>F. solani</i>	wheat	Kerman - Orozoieh

Parts of nuclear DNA was amplified using the above primers (ef1,ef2). The sequencing of the amplified using primers ef22.

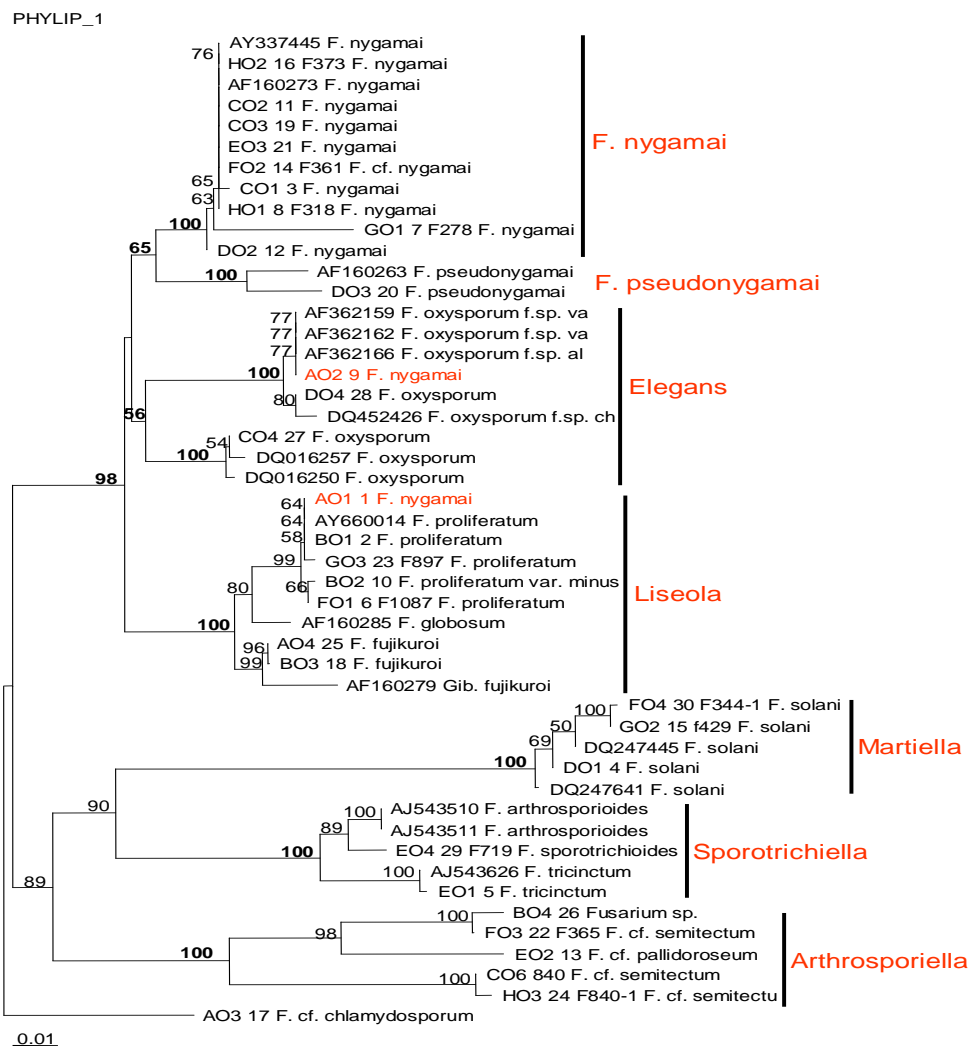
TEF gene sequences of length

To investigate the possibility of using a part of bases TEF gene for differentiation of *Fusarium* species, some parts were amplified using two primers ef1 and ef2. The equivalent size was amplified of 700 bp (Figure 2).

The sequence was amplified using primers ef22. Sequence length of approximately were 420-450 bp fragment sequences in most isolates and isolates Fo4-30 and 416 and 474 bp Do1-4, respectively.

Alignment and phylogenetic analysis

Align the sequences indicated that there are diversity and differences among sections and isolates of *Fusarium*. Neighbor-Joining methods of phylogenetic analysis and species, *F. chlamydosporum* perform as outgroup and phylogenetic tree was constructed (Figure 4).



Phylogram 1: Phylogenetic tree of the TEF gene of *Fusarium* isolates Neighbour-Joining method with thousand bootstrap replicates

In phylogenetic analysis was used as an outgroup *F. chlamydosporum* and other *Fusarium* species were several groups that were partially harmonized with morphological groups. *Elegans* section isolates completely separate group formed monophyletic group and isolates (Ao2-9) F-492 related to *F. nygamai* was also in this group. Isolates related *Liseola* were in tow separate and close groups to each and isolate of (Ao1-1) F-373 related to *F. nygamai* in this group, and close to *F. proliferatum* species and other isolates of *F. nygamai* were in a separate group. Isolates *F. pseudonygamai* were in a close group of *F. nygamai*. For compare the data from this research with the existing data in gene bank, the sequence of some of the *Fusarium* genus cheeks to gene bank and the analysis data. Data analysis and phylogenetic tree was drawn by the tested isolates were identified in seven groups. Nine isolates with

two isolates from the gene banks were in *F. nygamai* and isolates Do3-20 with a strain from the gene bank were in *F. pseudonygamai*. Two isolates with isolates Ao2-9 that were diagnosed morphologically *F. nygamai* were in Elegans group. Ao1-1 with six isolates that morphologically were diagnosed *F. nygamai*, were in Liseola group. Also three isolates in group Martiella and two isolates in Sporotrichiella were respectively. The five isolates of morphological differences with *F. semitectum*, were in Arthrosporiella that they need of more study.

Discussion

In this study, the use of molecular data in the differentiation of species and Phylogenetic relationships among species of this genus, part of the DNA of the TEF gene of the 30 genus *Fusarium* separation from Iran were amplified and sequenced. Genetic analysis of TEF sectors grouped in several different ways. The group with a few exceptions, the perfect harmony of the sections and groups are identified based on morphological characteristics. DNA sequence analysis of the isolates revealed consistent morphological characters and phylogenetic species to species as isolates of *F. nygamai* with slight differences in the two clusters and one group of *F. pseudonygamai* were in a close group to group *F. nygamai*. (Waalwijk colleagues ,1996) based on sequences of ITS1 and ITS2, DNA ribosomal *Fusarium* species sections Elegans, Liseola and Dlamia were compared with the results of ITS1 cluster showed more closely resembles other species of *F. beomiforme* and *F. polyphialidicum* showed both groups of species based on ITS sequences of the two clusters were not coincide with the boundaries of wards. The TEF gene is suitable for phylogenetic studies of this group. Based on data analysis of isolates of *F. oxysporum* isolates were in a group except Ao2-9 belonging to *F. nygamai*. Ao2-9 isolates of *F. nygamai* morphological characteristics were diagnosed within the group of Elegans and this proves that these isolates because the same is morphological characteristics *F. nygamai* and was the phylogenetic group of *F. oxysporum* therefore may be a new species that isolated of group *F. oxysporum*. Also, that the species *F. nygamai* from ways, such as chlamydospores of *Fusarium* species are similar to the Elegans and based on the results of studies conducted by other researchers and our results in this study, this species is close to *Fusarium* species in the section Elegans. Since only one gene has been used in this study and this gene is suitable for the study Some groups, including *F. oxysporum* and *Gibberella fujikuroi* species (O'Donnell et al., 2004; O'Donnell et al., 2000) and it is possible not appropriate to study other groups such as *F. nygamai* and this gene is not enough to study phylogenetic alone and also data can be used from other genes and other sectors DNA. (O'Donnell and colleagues ,1998c) was isolated evolutionary relationships of isolates Banana Panama disease *Fusarium oxysporum* f.sp. cubense from pathogenic strains of non-pathogenic strains from other hosts and comparison of DNA sequences of nuclear and mitochondrial genes studied and the results indicated that two taxa have five generation line different chromosomes in *F. oxysporum* f.sp. cubense. The results of this study showed that strains of *F. oxysporum* in a group close to the group were Liseola. Molecular relationships of fungi was studied within the group *Fusarium redolens*-*F. hostae* using a combination sequences of genes EF-1 α and mtSSU-rRNA and the results showed that *Fusarium redolens* - *F. hostae* such as a sister group of the group *G. fujikuroi* gene that was different being. Also, last group that is including two unknown *Fusarium* species, has similarity to the *F. oxysporum* and *F. nisikadoi*-*F. miscanthi* morphologically (Baayen et al., 2001). Based on molecular data analysis, isolates related to Liseola into two groups, which emanate from a sibling, indicating that the phylogenetic relationships of these species is near, Although there are differences between these species and are in several clusters. (O'Donnell and colleagues ,1998a) has been studied molecular systematic phylogeographic the *Gibberella fujikuroi* species groups using gene sequence of β -tubulin, mitochondrial small subunit ribosomal DNA and part 5'-28S based on information from the gene sequence, were located from three groups (clade). So American group were as a monophyletic and combined as the sister group Africa – Asia. Results of the analysis of genes (28S DNA, ITS, mtSSU, β -tubulin) and EF-1 α gene and calmodolin showed species group *G. fujikuroi* two major branches of ITS (ITS1 and ITS2) were revealed from two different ancestral origins. Phylogenetic analysis the combination of genes indicated that ten new species was identified (O'Donnell et al., 2000). TEF bases of sequence analysis showed that *F. nygamai* despite morphological differences between these species in the Elegans and Liseola and within this group, and this reflects the divergence of these species. However, given that the species *F. nygamai* some ways of producing the same species in the conidial chains are Liseola according to research conducted by other researchers, and the results, as described in the relevant sections of the species were Liseola and Elegans. (Young-mi and colleagues ,2000) were studied Genetic relationships among 12 species of *Fusarium* including sections of Martiella, Dlamia, Gibbosum, Arthrosporiella, Liseola, and Elegans using RFLP and PCR-based ribosomal DNA sequencing by seven restriction enzyme and the results showed that there was little difference between the size of the cut pieces of the five species and 12 isolates belonged to RFLP been particularly Martiella separately were into three RFLP and *F. solani* f. sp. piperis was surprising in species belonging to the section Elegans. As phylogram sequence analysis of isolates and separated them and recommended further studies are necessary to investigate the phylogenetic relationships among species from other parts of DNA and other species of this genus *Fusarium* is used and works

(O'Donnell and colleagues ,2000, 1998a and Steenkamp and colleagues ,2002) also confirms this divergence. (O'Donnell and colleagues ,1998a) to study the evolutionary relationship between isolates of *F. oxysporum* f.sp. *cubense* and compared non-pathogenic strains with their used five lineage. However, a discrepancy is created between morphological and phylogenetic implications of these studies have important implications in the classification of fungi, especially those that are in the *Gibberella fujikuroi* species. As the results of the phylogenetic species group *G.fujikuroi* and related species occur (O'Donnell and Cijelnik, 1997). Thus, the actual classification is very difficult to classify these fungi specially *Liseola* such problems have been reported by other researchers (O'Donnell et al., 1998a). (O'Donnell and colleagues ,2000) *Gibberella fujikuroi* was confirmed species groups phylogenetic relationships using DNA sequence amplified by PCR from four regions (28S rDNA, ITS, mitSS rDNA and β -tubulin) have already been studied was associated with two genes encoding proteins of the core of EF-1 α and calmodulin and their combinations studied, and the results obtained from the analysis of sequences of ITS ribosomal two regions of the gene ITS non-homologous and 10 species identified novel in three specific distribution and two species, respectively, in Asia, four in Africa and four in Latin America showed that a single ancestor (monophyletic) of *F. subglutinans* and its special form *F. subglutinans* f.sp. *pini* and *F. subglutinans* f.sp. *ananas*. The results also showed that this is an unrealistic classification and needs a classification of the real of the section. Results of molecular data showed that the isolates belonged to *F. solani* were identified in a group and there are differences between species, indicating that species differences within these groups. Molecular phylogenetic groups *Necteria haematococca-Fusarium solani* species using information from gene sequencing 28S rDNA, ITS and EF-1 α were studied by(O'Donnell ,2000) and the results showed that, although distinctive homogenates were clearly apparent mismatch or inconsistency between the information of 28S rDNA and ITS data showed, however, indicate that the combination of the introduced genes, they identified 26 species within the group showed that 15 heterothallic species, seven species and four species were homothallic. Also Phylogenetic relationship of *Fusarium solani* f.sp *phaseoli* and *Fusarium* agent soybean disease syndrome (SDS) were investigated using ribosomal DNA sequencing and RFLP markers for 24 isolates of the fungus. The results of a single ancestral confirmed species strongly and also DNA sequence data and a introduced primer can multiply SDS strains isolated from infected plants specifically (O'Donnell AND Gray, 1995). The differences within the groups so *Fusarium solani* was confirmed by other researchers, as studied by (Li and colleagues ,2000) between *F. solani* f.sp. *glycines* in soybean disease syndrome than *F. solani* isolated from other hosts with mitochondrial small subunit ribosomal DNA sequences by using the analyzed results showed that all isolates were in three distinct groups and *F. solani* f.sp. *glycines* is quite distinct and separate group with a shorter evolutionary history than other strains that are *F. solani*.

Isolates belonged to the species *F. tricinctum* and *F. sporotrichioides* two clusters were identified in a group, as *F. arthrosporioides* with *F. sporotrichioides* were in a cluster and *F. tricinctum* were indicating in a cluster close to *F. sporotrichioides* that the morphological differences is confirmed between these species in terms of the molecular differences between the two species. Results of molecular data indicate that the species *F. sporotrichioides* species *F. arthrosporioides* homology affinity than is *F. tricinctum*. (Yli-Mattila and colleagues ,2004) were studied using morphological and molecular differences between *Fusarium* species in Finland and northwestern Russia using morphological characteristics and sequence composition β -tubulin, IGS and ITS and the results showed that the group of European *Fusarium arthrosporioides* is a part from a group *F. avenaceum* and *F. tricinctum*, if the two species *F. avenaceum* and *F. arthrosporioides* that morphologically similar and are often confused with each other. Although molecular studies also were confirmed relatively close relationship of these species in a group. The results of this study isolates Ho3-24 and Co6-840 and Bo4-26 and Eo2-13 that are *Arthrosporiella* group differences in morphological characters such as colony color, conidial size and the lack of chlamydospores of *F. semitectum* sequence similarity and gene bank in search of the isolates of *F. semitectum* unconfirmed and based on personal correspondence with Mr. Gryfnhan other genes and other segments of DNA. (Steenkamp and colleagues ,2002) studied profile of morphological, biological and phylogenetic isolates of *F. subglutinans* using DNA sequences of genes β -tubulin, histon H3 and calmodulin the biological species concept and the phylogenetic results showed disagreement between the *F. subglutinans* in both the generation and analysis of the data indicated that there was a secret species within the species *F. subglutinans*. It seems to be a new species isolates that require further investigation and use of DNA information to other parts. The high similarity of the isolates showed the close relationship they had with them in a way that upholds.

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